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Report

On the Origin of a Novel Parasitic-Feeding Mode within Suspension-Feeding Barnacles

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Summary

In his monograph on Cirripedia from 1851 [1], Darwin pointed to a highly unusual, plateless, and most likely parasitic barnacle of uncertain phylogenetic affinity. Darwin's barnacle was *Anelasma squalicola*, found on deep-water sharks of the family Etmopteridae, or lantern sharks [2]. The barnacle is uncommon and is therefore rarely studied. Recent observations by us have shown that they occur at an unusually high prevalence on the velvet belly lantern shark, *Etmopterus spinax*, in restricted fjord areas of western Norway. A phylogenetic analysis based on ribosomal DNA data (16S, 18S, and 28S) from 99 selected barnacle species, including all available pedunculate barnacle sequences from GenBank, shows that *A. squalicola* is most closely related (sister taxon) to the pedunculate barnacle *Capitulum mitella*. Both *C. mitella* and species of *Pollicipes*, situated one node higher in the tree, are conventional suspension feeders from the rocky intertidal. Our phylogenetic analysis now makes it possible to establish morphological homologies between *A. squalicola* and its sister taxon and provides the evolutionary framework to explain the unprecedented transition from a filter-feeding barnacle to a parasitic mode of life.

Results and Discussion

In his authoritative monograph on barnacles from 1851 [1], Darwin investigated a very unusual plateless stalked barnacle with rudimentary mouthparts and highly reduced feeding appendages, or cirri. Darwin's barnacle is the infrequent and rarely studied *Anelasma squalicola*, which parasitizes various sharks of the family Etmopteridae [2]. This species is a likely candidate for an extant "missing link" in the evolutionary transition from suspension feeding toward attaining a parasitic lifestyle.

The parasitic *A. squalicola* is found embedded in the skin of its shark host (Figures 1A and 1C). Although fully equipped with six pairs of cirri (thoracic appendages), used for suspension feeding in normal barnacles, these are no longer used for food capture in *A. squalicola* (Figure 2A). Instead, the barnacle has embedded a root-like body part into the flesh of the shark that serves both as an anchor for the parasite and as a feeding device (Figure 1B, 1C, and 2A), able to transfer nutrition from the shark to the barnacle [2–4]. Thus, *A. squalicola* has just passed the pivotal evolutionary point at which it has abandoned conventional suspension feeding and irreversibly adopted a novel parasitic lifestyle, while still retaining clear morphological similarities to its suspension-feeding barnacle relatives (Figure 2). Very little information exists about the evolutionary transition from a free-living organism to parasitism. Many animal taxa contain species that have developed a parasitic lifestyle, but these are fully parasitic and thus have not retained any of the characters found in their free-living relatives. This highlights the uniqueness of *A. squalicola* and the insight it can provide on the evolution of parasitism.

Parasitism in Thoracican Barnacles

Of the about 1,000 extant species of stalked and acorn barnacles (Cirripedia: Thoracica), only a few genera are true parasites, defined as a long-lasting bond between two species in which one is benefiting from, and harmful to, the other. But only *Anelasma* parasitizes a vertebrate host [5]. This is surprising because many stalked and acorn barnacles are epibiotic suspension feeders, living on various marine animals, including vertebrates such as turtles, sea snakes, sea cows, and especially whales. Hence, one would imagine that more barnacle species would have developed methods to utilize the virtually endless food resource (tissue and blood) available just a few millimeters below the attachment site of the barnacle. Barnacles are generally well represented in the fossil record due to the protective calcareous plates that surround the adult [5]. However, the fossil record is obviously patchy, and there are reduced chances of preservation of plateless barnacles. Nevertheless, no fossil barnacles have been put forward as putative parasites on vertebrate hosts, and *Anelasma* remains unique among the large number of extant taxa in exhibiting such a mode of living [6]. Several of the numerous species of whale barnacles are also deeply embedded in the dermis of their host and often have a much-reduced armature of shell plates, but none of them have been reported to feed on their hosts [7].

Phylogenetic Position of *Anelasma*

Figure 3 shows a phylogram from a Bayesian analysis of the data set, revealing a long *Anelasma* branch proportional to a large amount of DNA substitutions. To examine whether this long branch causes attraction to the outgroups or other long branches in the phylogeny, we divided our data sets into single genes and applied the testing regime suggested by Bergsten [8]. This involved multiple analyses after serial removal of the closest sister group to the suspected long branch, and also removal of the long branch itself, to assess impact on the positions of these taxa in the overall phylogeny. Other analyses involved removal of outgroup taxa to test for any influence on positions of long-branch taxa. Our analyses did not indicate signs of long-branch attraction, and we consider the phylogenetic position of *Anelasma*, as sister group to *Capitulum mitella*, as well founded.

To understand the evolutionary forces that enabled *A. squalicola* to make the transition from suspension feeding to parasitism, a comparison between *A. squalicola* and its closest nonparasitic relatives is an obvious starting point. In

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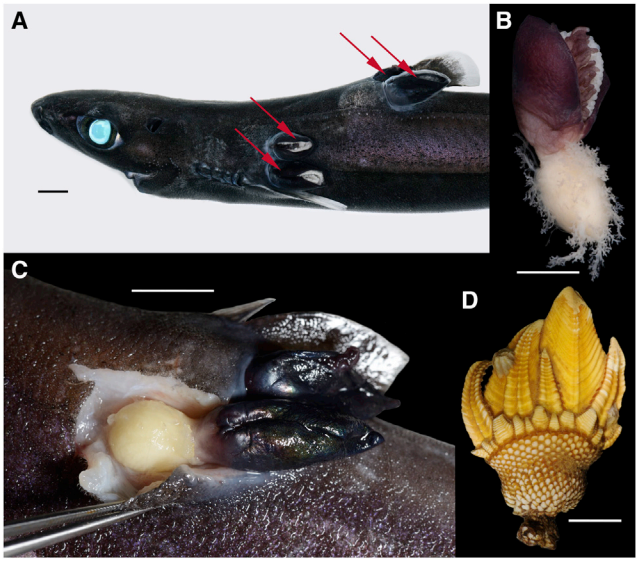

Figure 1. *Anelasma squalicola* In Situ on *Etmopterus spinax* and Comparison with the Closest Extant Relative, *Capitulum mitella*

(A) The velvet belly lantern shark, *Etmopterus spinax*, infested with two pairs of *Anelasma squalicola* (red arrow heads), two in front of the first dorsal fin and two above the pectoral fin.
(B) *A. squalicola* in total showing the capitulum and the exposed peduncle.
(C) A specimen of *A. squalicola* (in situ) with the peduncle partially dissected out from the flesh of the shark.
(D) *Capitulum mitella*, the East Asian intertidal barnacle, which is the closest living relative to *A. squalicola*.
Scale bars represent 1 cm (A, C, and D) and 0.5 cm (B).

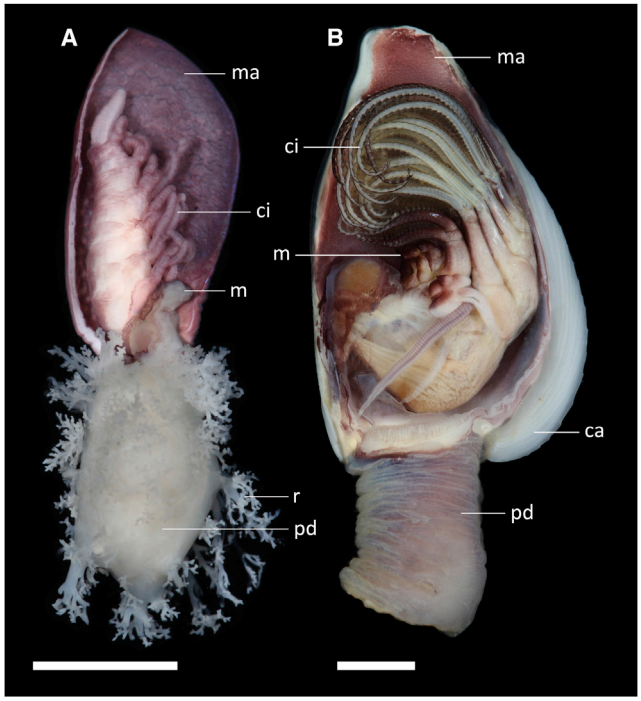

Figure 2. Anatomical Comparison of *Anelasma squalicola* and a Typical Filter-Feeding Barnacle

Comparison of *Anelasma squalicola* and *Lepas* sp., both with the part of the mantle (ma) facing the viewer removed.
(A) *A. squalicola*. The whitish lower half constitutes the modified peduncle (pd), carefully dissected free of the muscle tissue of its shark host. The delicate rootlets (r) protruding from the peduncle are believed to increase the surface area, making the uptake of nutrition from the shark more efficient. The upper part of the body shows the thorax of the barnacle within the cavity of the mantle (ma). Note the reduced cirri (ci) and mouth (m) compared to *Lepas*.
(B) *Lepas* sp. The thorax is exposed within the shell plate covered mantle (ma) with well-developed, functional cirri (ci) and mouth (m). The thick, unpaired shell plate Carina (ca) protects the dorsal side of the barnacle. The peduncle (pd) is used to attach to floating objects.
Scale bars represent 0.5 cm.

our analysis, *A. squalicola* is unambiguously placed deeply within the suspension-feeding barnacles (Thoracica) as sister group to *Capitulum mitella*, a pedunculate barnacle from the rocky intertidal, which is common and commercially utilized (edible) in East Asian waters (Figure 1D). The present phylogeny (Figure 3) largely agrees with previous analyses based on molecular data sets [9–11], although it differs slightly in the relative position of *Pollicipes* and *Capitulum*. Both our analysis and that of Perez-Losada et al. [10] agree in having the pedunculate barnacles *Pollicipes*, *Capitulum*, and *Lithotrya* as closest relatives to Sessilia (Verrucomorpha and Balanomorpha). *Anelasma* has not previously been included in a phylogenetic analysis, either by morphological or molecular data. Existing taxonomy [12] places it in the Heteralepadomorpha, which consists of pedunculate forms without any shell plates. This has led to the inference that this group might represent the earliest clade on the thoracican or even the cirripede tree before mineralization evolved in the cuticle [13, 14]. However, our phylogeny including *Anelasma* shows that this taxon is nothing but a polyphyletic assemblage of pedunculate cirripedes that have secondarily lost all shell plates, probably due to being epibiotic on a variety of other organisms.

Adaptation to Parasitism

The majority of barnacles other than the Sessilia have a fleshy stalk (peduncle) that elevates a calcareous shell (capitulum) from the surface of the settlement site. The position of *A. squalicola* as sister taxon to *C. mitella* therefore strongly suggests a homology between this stalk and the device that *A. squalicola* uses to anchor itself in the flesh of the host and that most likely also acts as the sole trophic organ of the parasite. The cirri (thoracopods) in *Anelasma* are all present as six biramous pairs, but unlike other thoracicans, they are completely lacking filter setae (Figure 2) and thus seem wholly incapable of performing suspension feeding. Although *A. squalicola* does retain an alimentary canal with an open mouth and anus [15], it is unlikely that the adult barnacle is capable of feeding that involves the alimentary tract since it was found to be empty in all specimens examined. *A. squalicola* is devoid of shell plates, but in this feature it resembles several other thoracican species, all of which are epibiotic on other organisms. In addition, species of *Octolasmis*, *Conchoderma*, and several whale barnacles have a highly reduced shell plate armature, again correlated with an epibiotic mode of life [16]. The parasitic nature of *Anelasma* also raises interesting problems with respect to reproduction. The species is clearly hermaphroditic, since all individuals possess a penis and dwarf males are not present. It therefore appears that they use their penis for copulation with an individual sitting nearby as in other hermaphroditic thoracicans. In agreement with this, *Anelasma* is usually found sitting in pairs or more side by side (Figure 1A). This raises interesting questions about how the cypris larvae manage to not only

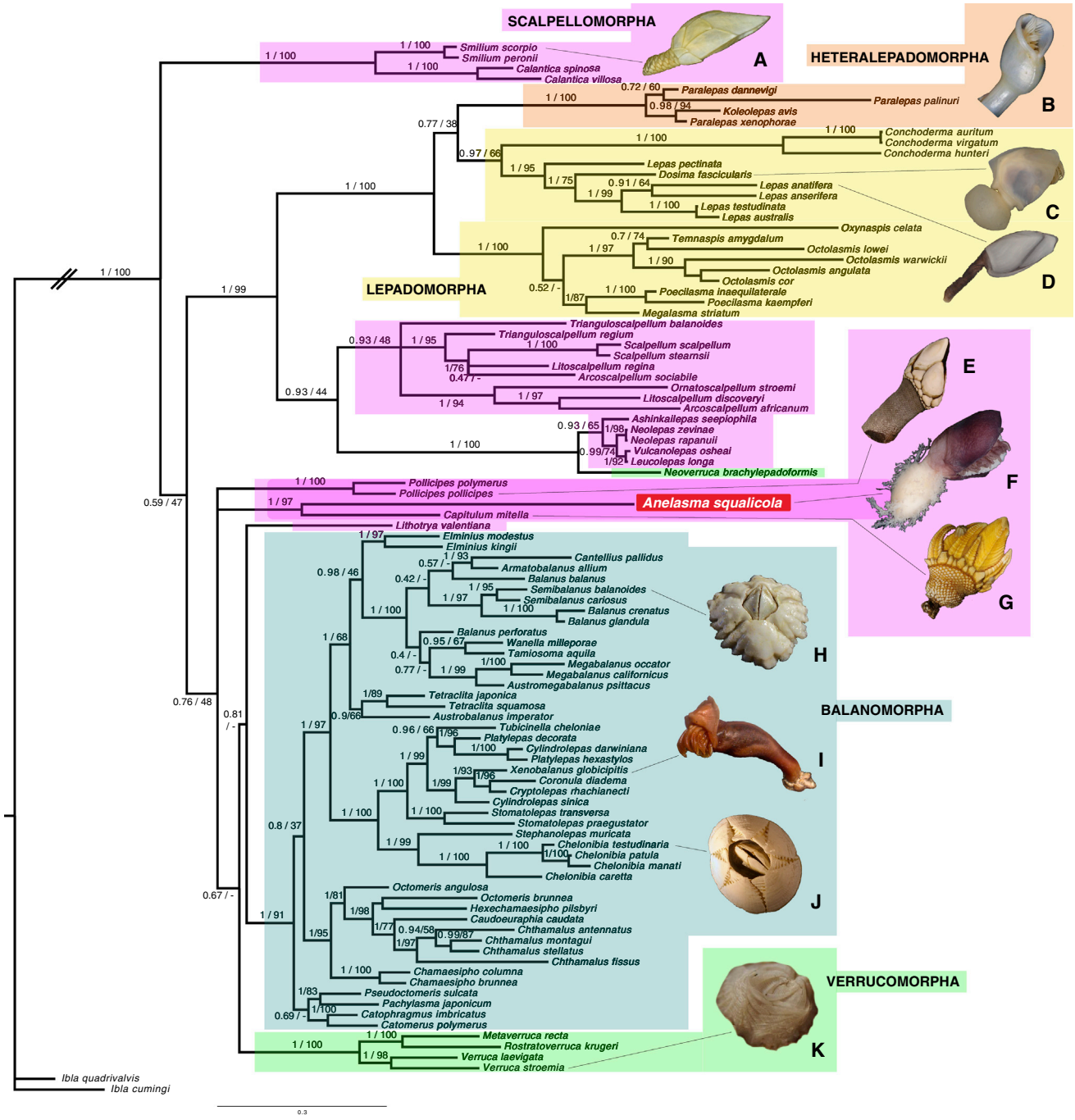

Figure 3. Evolutionary Relationships of *Anelasma squalicola* and Other Barnacles

Bayesian phylogenetic tree for the three-gene concatenated data set with color coding indicating suborder groupings and the position of *Anelasma squalicola* (marked in red). Nodal support is indicated in the form of Bayesian posterior probabilities (PP) and maximum-likelihood bootstrap values; nodes with PP values $\leq$50 have been collapsed (see the Supplemental Experimental Procedures available online for details). Representative morphology of major cirripede groups is illustrated by (A) *Smilium scorpio*, (B) *Paralepas* sp., (C) *Dosima fascicularis*, (D) *Lepas anatifera*, (E) *Pollicipes pollicipes*, (F) *Anelasma squalicola*, (G) *Capitulum mitella*, (H) *Semibalanus balanoides*, (I) *Xenobalanus globicipitis*, (J) *Chelonibia testudinaria*, and (K) *Verruca stroemia*. See also Table S2.

locate their shark host, but also to find and settle next to a conspecific.

Parasitism and Epibiosis in Other Cirripedes

While epibiosis is very common, parasitism is not and has only evolved few times in Cirripedia. The Cirripedian suborder Rhizocephala comprises more than 250 species of parasitic barnacles, all of which infest crustaceans and are so modified that they can only be recognized as barnacles, or even as crustaceans, by means of their larvae [6, 17]. The adults are reduced to a sac containing the reproductive apparatus and the trophic organ, a root system, which extracts nutrition from the hemolymph of the host. These taxa form a monophyletic sister clade to the Thoracica, and all have separate sexes

Table 1. Fossil Calibration and Divergence Time Estimation

Species	Geological Age	Node	Divergence Time
Praelepas jaworskii	306.5–311.7 [5]	A	311.96 (306.30–322.92)
Pollicipes aboriginalis	83.5–85.8 [24]	B	87.59 (83.42–95.90)
Pachydiadema (*Catophragmus*) *cretacea*	70.6–89.3 [24]	C	108.35 (83.20–133.27)
Verruca tasmanica	70.6–85.8 [24]	D	74.64 (70.45–83.13)
Palaeobalanus lindsayi	37.2–48.6 [24]	E	90.16 (68.98–111.64)
Chamaesipho brunnea	16–23 [24]	F	20.91 (16.00–29.81)
Anelasma-Capitulum			126.50 (52.39–196.93)

Fossil taxa and ages (Ma) used for calibrations in BEAST divergence time estimates, which are presented with corresponding 95% highest posterior density values together with the estimated time to most recent common ancestor for *Anelasma-Capitulum*. Positions of calibrated nodes are indicated as points A–F in Figure 3. All calibrations were introduced as minimum node ages using exponential prior distributions, with the offset adjusted to correspond to the fossil age and a soft upper bound to allow for possible uncertainty in the fossil record.

with dwarf males situated deep within the female body [17]. Within thoracicans, however, the only other parasite using a root-like feeding system is the polychaete-infesting *Rhizolepas*, with only two described species [18, 19]. *Rhizolepas* is considerably more modified to parasitism than *Anelasma*. It also has a trophic organ inside the host, but in *Rhizolepas* it is profusely branching as in rhizocephalan barnacles, and on the external body part both the mantle and the cirri are much reduced. It lacks a mouth and an anus, although the alimentary canal as such is retained [18]. As in *A. squalicola*, *Rhizolepas* also sports a posteriorly sited penis, indicating that the species is hermaphroditic. A few other epibiotic thoracicans parasitize on their host animals, but in a rather predatory mode, feeding via the mouth. *Koleolepas*, epibiotic on sea anemones, uses its cirri to chip off pieces of the host tentacles when they withdraw after capturing food [20]. The balanid barnacle *Pyrgoma monticulariae*, which inhabits corals, has reduced cirri and mouthparts adapted to scrape away coenenchyme from the overgrowing coral [21]. Within the genus *Pyrgoma*, a gradation of species exists from wholly parasitic to wholly filter feeding. The morphological trend toward parasitism, represented by the species of *Pyrgoma*, provides a parallel evolutionary path toward parasitism, although the "peduncle-feeding" mechanism in *Anelasma* is fundamentally different from the feeding found in any of the *Pyrgoma* species.

Whale barnacles form a monophyletic group of acorn barnacles [22] in which the body can be deeply embedded in the dermis of their cetacean host, but they retain fully functional cirri and obtain all their nourishment by suspension feeding. This mode of life has entailed some reductions in the shell plates, and in *Xenobalanus* this has gone so far that opercular plates are missing and the existing plates are used to anchor the very elongated body in the cetacean tissue. This body shape, seemingly rather similar to *A. squalicola*, caused Darwin [1] to discuss whether the latter might not in fact be a balanomorphan, related to the whale barnacles. But with true insight, he argued that *A. squalicola* has features such as a true peduncle and special egg anchoring devices (frena) never seen in balanomorphans. He thereby tacitly dismissed a common evolutionary origin of epibiosis or parasitism in these two forms. Although evolution was never explicitly discussed in his four barnacle volumes, his correspondence with, e.g., Hooker [23] bears evidence that he in fact used barnacles as a testing ground for his "origin of species theory."

Evolution of Parasitism

Anelasma is the only barnacle (Cirripedia) infesting a vertebrate. Its relationship with suspension feeding, pedunculated barnacles from the rocky intertidal may seem surprising. A priori, one might have expected barnacles parasitizing vertebrates to have evolved from acorn barnacles that are epibiotic on whales, because these already have their base deeply embedded within the dermis of the cetacean host. This is a far more complicated relationship than in their close allies, the snake and turtle barnacles, which attach on the scales and carapaces of their hosts [22]. Neither *Capitulum* nor its close relative *Pollicipes* lives in habitats frequented by the hosts of *Anelasma*. This raises the possibility that the *Anelasma* lineage diverged long ago and may represent the only extant representative of a previously more speciose clade whose members originally were conventional filter feeders and only recently made the transition to parasitism. This fits well with our data suggesting that the divergence between *Capitulum* and *Anelasma* happened in the Mesozoic around 120 million years ago (Table 1). Furthermore, the available fossil data also puts the divergence between the *Capitulum*, *Pollicipes*, and Sessilia lineages more or less in the same time frame (Figure 4).

The morphology of the cirri in *Anelasma* indicates that they are highly reduced due to being largely or wholly nonfunctional. Not only do the cirri have a rudimentary appearance compared to other filter-feeding barnacles (Figure 2), but an initial assessment of the cirral buds in *Anelasma* indicates that a large percentage of individuals exhibit clear asymmetries, unrelated to attachment position on the host (unpublished data). This indicates that the usually highly symmetrical and sophisticated cirri in filter-feeding barnacles are no longer subject to positive selection pressures in *Anelasma*. Preliminary data from stable carbon and nitrogen isotopes further indicates that the nutritional source of the barnacle is the host and not planktonic food items.

We conclude that *Anelasma* has evolved from a suspension-feeding, pedunculate barnacle ancestor and is unique in having made the transition between this mode of life and parasitism. The information provided here enables us to understand which morphological, physiological, and genetic traits are, at a minimum, required to successfully embark on a parasitic lifestyle on a vertebrate host—and thereby to address some of the questions that puzzled Darwin more than 150 years ago.

Experimental Procedures

Data assembly and Phylogeny Estimation

This study includes data covering the widest possible range of extant cirripede taxa, comprising new data for *Anelasma squalicola* and *Dosima fascicularis* together with extensive ingroup and outgroup sampling from GenBank. GenBank accession numbers for new sequences are KF781341–KF781346; further details of new sequences and data selection criteria, along with all other GenBank accession numbers, are described in detail in the Supplemental Experimental Procedures accompanying this article.

Parallel data sets were generated for each gene, with one set analyzed using Gblocks to identify hypervariable portions of the alignments, which were subsequently removed, and the other fully retained. Best-fit models of nucleotide substitution were inferred for each gene and treatment, as well as for the combined data set, and applied in maximum-likelihood and

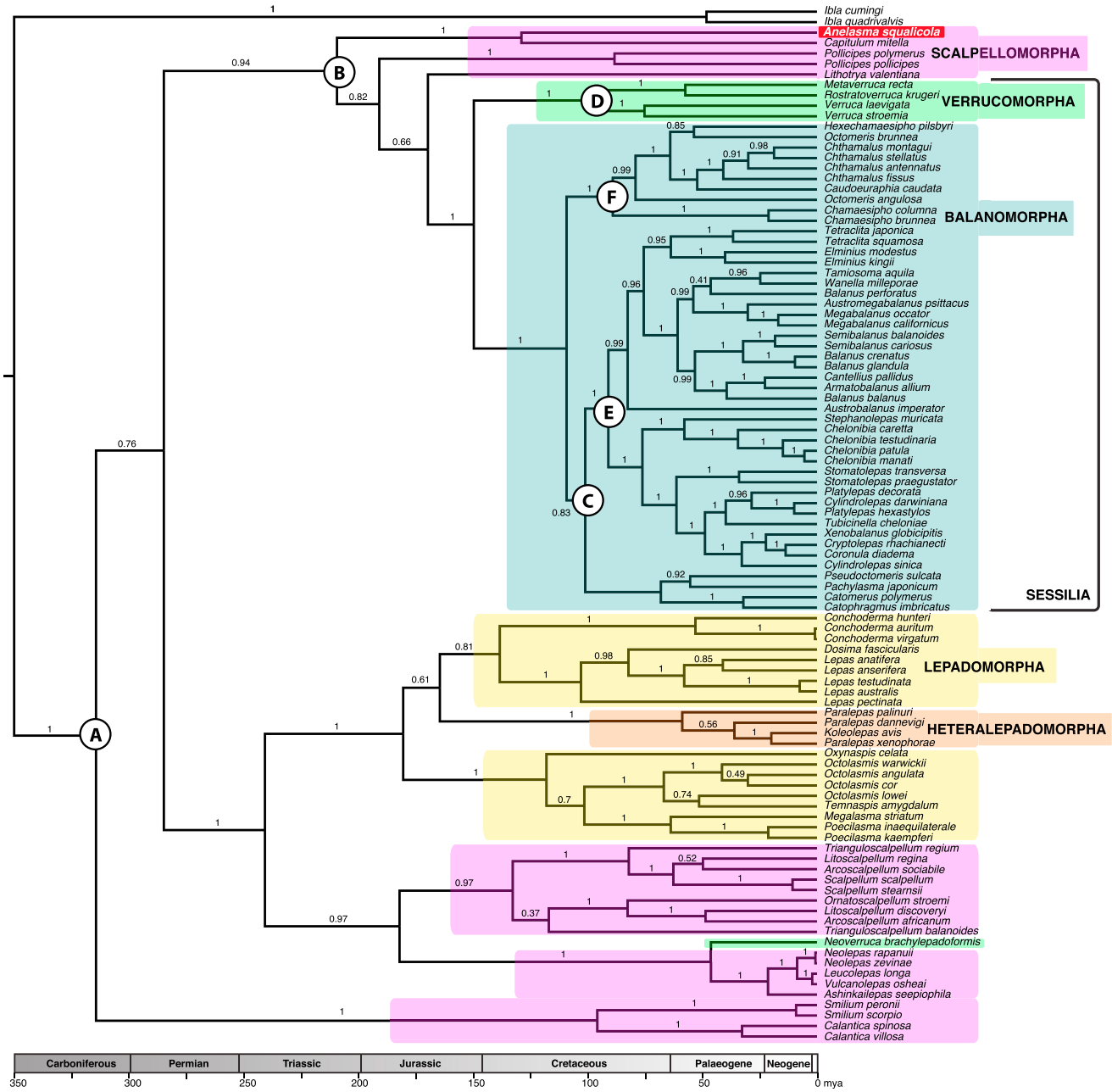

Figure 4. Divergence Time Estimates for *Anelasma squalicola* and Major Barnacle Groups

Results of Bayesian analyses (BEAST) of divergence time estimates for cirripede taxa, with color-coded groupings of taxa into suborders as in Figure 3. Clade posterior probability support values are shown for all nodes. Dating calibration nodes are indicated by the letters A–F, corresponding to fossil taxa listed in Table 1. A simplified geological time scale is presented, scaled according to the date estimates from the BEAST analyses. See also Tables S2 and S3.

Bayesian analyses. Comparisons of the resulting topologies indicated broad agreement and all final analyses were performed on the full concatenated data set (for full details of all analyses, see the Supplemental Experimental Procedures).

Fossil Data and Date Estimation

Estimation of divergence times was performed in BEAST using the final multigene Bayesian topology as a starting tree (see the Supplemental Experimental Procedures for details). Six fossil taxa were used as calibration points in the tree, with a log-normal relaxed clock methodology allowing for lineage-specific rate heterogeneity. Minimum fossil ages ranged from 16 to 306.5 Ma, and exponential date priors with soft upper bounds were employed in order to reflect possible uncertainty associated with fossil calibrations. Details of fossil taxa and locations of calibration points are presented in Table 1 and Figure 4.

Accession Numbers

The GenBank accession numbers for the new sequences reported in this paper are KF781341–KF781346.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.05.030.

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